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Effect of prenatal androgens on click-evoked otoacoustic emissions in male and female sheep (*Ovis aries*)

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ABSTRACT

Otoacoustic emissions (OAEs) were measured in male and female Suffolk sheep (*Ovis aries*). Some sheep had been administered androgens or estrogens during prenatal development, some were gonadectomized after birth, and some were allowed to develop normally. As previously reported for spotted hyenas, gonadectomy did not alter the OAEs for either sex; accordingly, the untreated/intact and the untreated/gonadectomized animals were pooled to form the control groups. The click-evoked otoacoustic emissions (CEOAEs) exhibited by the female control group ($N=12$) were slightly stronger (effect size=0.42) than those in the male control group ($N=15$), which is the same direction of effect reported for humans and rhesus monkeys. Females administered testosterone prenatally ($N=16$) had substantially weaker (masculinized) CEOAEs than control females (effect size=1.15). Both of these outcomes are in accord with the idea that prenatal exposure to androgens weakens the cochlear mechanisms that underlie the production of OAEs. The CEOAEs of males administered testosterone prenatally ($N=5$) were not different from those of control males, an outcome also seen in similarly treated rhesus monkeys. Males administered dihydrotestosterone (DHT) prenatally ($N=3$) had slightly stronger (hypo-masculinized) CEOAEs than control males. No spontaneous otoacoustic emissions (SOAEs) were found in any ears, a common finding in non-human species. To our knowledge, this is the first ruminant species measured for OAEs.

Introduction

Otoacoustic emissions (OAEs) are sounds that are produced in the cochlea and propagate out through the middle-ear system into the external ear canal (Kemp, 1978, 1979; Probst et al., 1991; Shera and Guinan, 1999). An interesting feature of OAEs in humans is that they exhibit a large sex difference that exists in newborns (Strickland et al., 1985; Burns et al., 1992, 1994; Morlet et al., 1995, 1996) as well as in adults (Bilger et al., 1990; Talmadge et al., 1993; McFadden, 1993; McFadden et al., 1996; McFadden and Pasanen, 1998, 1999; McFadden and Shubel, 2003). The direction of the sex difference in humans is stronger OAEs in females than in males. Because this sex difference exists at birth, it has been proposed that exposure to high levels of androgens prenatally weakens OAEs by weakening the underlying cochlear mechanisms that contribute to them (commonly called the cochlear amplifiers; see Davis, 1983). This has been called the prenatal-androgen-exposure explanation (e.g., McFadden, 2002, 2008). Because strong OAEs are associated with good hearing sensitivity (e.g., McFadden and Mishra, 1993), the common interpretation is that strong OAEs and sensitive hearing both are results of a common cause — strong cochlear amplifiers.

OAEs of differing types have been measured in a wide array of species (Köppl, 1995; Martin et al., 1985, 1999). The results have revealed both similarities to and differences from the OAEs of humans, but unfortunately sex differences have not commonly been studied in the OAEs of non-humans. One species, rhesus monkeys, did exhibit sex differences similar to those seen in humans; namely, stronger click-evoked OAEs (CEOAEs) in females than in males (McFadden et al., 2006a). In addition, the CEOAEs for male rhesus monkeys showed a seasonal effect; the CEOAEs were weaker in the breeding season (Fall) and stronger in the non-breeding season, when androgen levels are high and low, respectively. Thus, if prenatal androgens are responsible for the sex differences in OAEs, there appear to be both organizational and activational effects of androgens on the CEOAEs of rhesus monkeys (also see McFadden, 2000).

Another type of OAE measured in those same rhesus monkeys (distortion-product OAEs or DPOAEs) exhibited a much smaller sex difference than did the CEOAEs (McFadden et al., 2006a). Examination of the DPOAE data reported by Torre and Fowler (2000) for rhesus monkeys also reveals only a small sex difference favoring the females, and only in the youngest animals (all of which were middle-aged). In humans, the sex difference in DPOAEs also is small (Gaskill and Brown,

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1990; Moulin et al., 1993; Cacace et al., 1996; Dhar et al., 1998; Bowman et al., 2000; McFadden et al., in press-b). Taken together, these facts suggest that CEOAEs are more affected by early developmental mechanisms of sexual differentiation (possibly hormonal mechanisms) than are DPOAEs.

By contrast, both the CEOAEs and DPOAEs were slightly *weaker* in female spotted hyenas than in males, not stronger (McFadden et al., 2006b). This is an extremely unusual mammalian species because female spotted hyenas are exposed to high levels of androgens during prenatal development and, as a consequence, they are highly masculinized in body, brain, and behavior (Frank et al., 1991; Glickman et al., 1992). The explanation offered for the absence of a human-like sex difference in the OAEs of this species was that the cochlear amplifiers of female spotted hyenas are weakened by the high levels of androgens to which they are naturally exposed prenatally (McFadden et al., 2006b). In accord with this explanation, we observed stronger CEOAEs in spotted hyenas of both sexes that were treated with androgen-blocking agents during prenatal development. An ideal test of the explanation, as yet not done, would be measurements of OAEs in another species of hyena, none of which has highly masculinized females.

Guimaraes et al. (2004) reported sex differences in CBA mice. Females had stronger DPOAEs than males, the same direction of effect as in humans and rhesus monkeys. However, because this sex difference was evident in middle-aged mice, but not in young mice, it may turn out to be an example of a differential, age-related hearing loss rather than an organizational effect of androgens. In chinchillas, S.L. McFadden et al. (1999) found no sex differences in DPOAEs even though females were more sensitive than males at high frequencies according to an evoked-potential measure obtained from a gross electrode implanted in the inferior colliculus. Valero et al. (2008) reported much larger DPOAEs in female than in male marmoset monkeys, a species for which little else is known about the auditory periphery. Only DPOAEs were measured in the Guimaraes et al., the S.L. McFadden et al., and the Valero et al. studies, not CEOAEs.

Here we report OAE measurements made on sheep of the Suffolk variety. To our knowledge, no previous measurements of OAEs in a ruminant species have been published. According to one report (Wollack, 1963), hearing sensitivity in sheep is between 10 and 40 dB worse than in humans over the range of about 0.1 kHz to 5.0 kHz, and it averages about 18 dB worse over the range of 1.0 kHz to 5.0 kHz where we measured CEOAEs; the best hearing is around 10 kHz; and hearing extends to at least 40 kHz. We are not aware of any explanation for why hearing would be so poor in the low frequencies and then extend to such high frequencies in this species, but Masterton et al. (1969) have argued that the audible frequency range in a species may be determined, in part, by its ability to use interaural level differences to localize sound sources in space. We know of no studies on sound localization in sheep.

Based on the known sex differences in the OAEs measured in humans and rhesus monkeys, we predicted that the OAEs of female sheep would be stronger than those of male sheep. Some of the sheep to be tested had been exposed to higher-than-normal levels of androgens or estrogens during the second trimester of prenatal development (see Bormann et al., submitted for publication; Roberts et al., 2008), providing us with additional tests of the effects of prenatal hormones on OAE strength. One prediction was that OAEs would be weakened in sheep treated with androgens prenatally. Some of the sheep tested had been gonadectomized within two weeks of birth and were not receiving supplemental hormones; based on our experience with spotted hyenas (McFadden et al., 2006b), we expected these post-natal manipulations to have little effect on OAE strength.

Many of our expectations were confirmed, but an unanticipated result also was obtained. Specifically, some sheep yielded strong CEOAEs but only quite weak, or no, DPOAEs. In the past, dissociations between different types of OAE have been observed (e.g., Wier et al., 1988; McFadden and Pasanen, 1994; Whitehead et al., 1996), but only in the context of short-term injury to the cochlea. Here, the presence of strong CEOAEs makes cochlear injury an unlikely explanation. Elsewhere (McFadden et al., in press-a) we discuss this dissociation between and DPOAEs and CEOAEs in sheep and describe some of the tests we made to document its existence. Here we describe only the CEOAE data, which appear to be unaffected by the dissociation from the DPOAE data.

Methods

All experimental procedures were performed in accordance with NIH guidelines and were approved by the Committees on the Use and Care of Animals at both the University of Michigan (UM) and The University of Texas (UT). The sheep lived outdoors in a natural environment at the Reproductive Sciences Program Sheep Research Facility (Ann Arbor, MI at 42° 18′ North latitude). After weaning, lambs had access *ad libitum* to alfalfa hay and pellets, pasture, and water. Females and same-aged, vasectomized males were housed together. OAE measurements were obtained on three multi-day visits of the UT team to Ann Arbor: in November and December 2006 and in May 2007.

The various groups of treated animals tested for this study were created by the UM team in order to study experimental questions about endocrine function and sex-typical behavior in sheep that arose in part from earlier work by the UM team. The auditory measurements reported here were simply added on to the existing protocol, and were implemented when it was convenient for both the UM and UT teams.

Subjects

The sheep tested (*Ovis aries*) were of the Suffolk variety. All of the female sheep were born between early March and early April 2005, and all of the male sheep were born between early March and early April 2006. Thus, the females were approximately 20–26 months of age and the males approximately 8–14 months of age at the time of OAE testing. The nominal gestational period for sheep is 21 weeks, infancy lasts about 2 weeks, and the pre-weaning period lasts about 8 weeks. The pre-pubertal period, as measured by the rising level of luteinizing hormone, lasts about 10 weeks in males and about 30 weeks in females. Measurable testosterone is present at about 4 months of age in males, and estrous cycles are present at about 7 months in females. The nominal lifespan of sheep is 8–13 years. Thus, all the sheep tested were young adults. Because some of the sheep had received special treatments during gestation, the data are presented separately for the different groups. The details of the groups are given in what follows, and the *N*s for the groups are shown in Table 1.

Table 1
Number of female and male sheep in the various groups

Group	Female	Male
Untreated	8	9
Untreated/GDX	4	6
Prenatal testosterone		
Non-obese	10	5
Obese	6	0
Prenatal estradiol (E_2)	2	0
Prenatal DHT	0	3

For this study, the untreated and untreated/GDX sheep were combined within sex to create the two control groups, and the non-obese and obese females were combined to create a single female prenatal-testosterone group.
GDX = gonadectomized.
DHT = dihydrotestosterone.
Untreated males were vasectomized (VX).